

Particle size determines the anti-inflammatory effect of wheat bran in a model of fructose over-consumption: Implication of the gut microbiota

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ABSTRACT

We investigated the impact of the particle size of wheat bran on gut dysbiosis and inflammation induced by a fructose overload. Mice received drinking water with or without fructose (30%) and a standard diet supplemented with or without 5% of wheat bran fractions characterized by different average particle sizes (1690 μm versus 150 μm) for 8 weeks. Fructose increased *Enterobacteriaceae* associated with higher expression of key inflammatory genes in the liver. The two wheat bran fractions differently affected specific gut bacteria known to be involved in the regulation of the gut barrier function and/or inflammatory processes. Moreover, wheat bran with small particle size was the sole fibre that reduced hepatic and systemic inflammatory markers upon high fructose intake. The anti-inflammatory effects of wheat bran may be dependent on their particle size and could be related to the changes in caecal *Enterobacteriaceae*.

1. Introduction

Consumption of refined sugars has increased over the past decades. In particular, the intake of sugars like fructose has increased substantially in the Western countries. This is largely through its use as a sweetener in beverages and in the diet with the production of “sweet corn-based syrups” known as “high-fructose corn syrups” (Bray, Nielsen, & Popkin, 2004; Cordain et al., 2005; Pereira et al., 2017). The overconsumption of fructose has been implicated as a contributing factor in the development of metabolic diseases such as in non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH) (Lim, Mietus-Snyder, Valente, Schwarz, & Lustig, 2010; Matteoni et al., 1999; O’Sullivan et al., 2014; Vila et al., 2011). Growing evidence supports that increased intestinal permeability participates in fructose-induced development of NAFLD and chronic inflammation. In fact, the acceleration of fructolysis in the intestine may cause local inflammation and

reduce tight junction proteins (such as occludin and zonula occludens 1 (ZO-1)) expression in intestine, leading to an increase in intestinal permeability (Zhang, Jiao, & Kong, 2017). Alteration in the gut barrier function results in the translocation of bacterial products like lipopolysaccharides (LPS) in the portal blood flow that leads to a condition defined as “metabolic endotoxemia” (Cani et al., 2008; Farhadi et al., 2008; Ruiz et al., 2007; Thuy et al., 2008). Furthermore, high levels of circulating inflammatory cytokines, which are often observed in fructose-fed animals or patients may impair intestinal mucosal integrity and increases metabolic endotoxemia (Zhang et al., 2017). The liver due to its anatomical links to the gut is continuously exposed to gut-derived endotoxins delivered via portal vein and thus functions as the body first line of defense (Mouzaki et al., 2013). LPS activates toll-like receptors (TLR) resulting in the stimulation of lipid peroxidation and the production of pro-inflammatory mediators such as tumor necrosis factor- α (TNF- α) or interleukin-6 (IL-6), and reactive oxygen species

Abbreviations: AMP, Antimicrobial peptides; AX, Arabinoxylan; CD, Clusters of differentiation; IFN- γ , Interferon gamma; IL, Interleukin; LPS, Lipopolysaccharides; MCP1, Monocyte chemoattractant protein 1; Nox1, NADPH oxidase 1; NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; ROS, Reactive oxygen species; TLRs, Toll-like receptors; TNF- α , Tumor necrosis factor alpha; WBs, Wheat bran with reduced particle size; WB, Wheat bran; ZO-1, Zonula occludens-1

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(ROS) which have been implicated in the development of liver inflammation and fibrosis (Spruss et al., 2009). In particular, fructose-induced endotoxemia activates Kupffer cells via TLR4, which may be partially involved in the development of NAFLD, and subsequently trigger nuclear factor-kappa B (NF- κ B) activation and TNF- α overproduction (Zhang et al., 2017). Meanwhile, ROS production also participates in endotoxin-dependent development of NAFLD. Elevation of plasma LPS and TNF- α levels of animals with high fructose diet, are blunted by treatment with antibiotic or inoculation with faecal samples from control donor rats (Di Luccia et al., 2015). Moreover, growing evidence in this context suggests that the onset of fructose-induced NAFLD is associated with the development of small intestine bacterial overgrowth and gut microbiota alteration, and is counteracted by antibiotic treatment in mice (Bergheim et al., 2008; Payne, Chassard, & Lacroix, 2012; Sabate et al., 2008; Schnabl & Brenner, 2014; Shanab et al., 2011). These findings suggest that the intestinal microbiota is one of the key components in the progression of NAFLD and hepatic inflammation (Le Roy et al., 2013; Payne et al., 2012; Spruss et al., 2009). More recently, it has been shown that high intake of fructose in humans (40 g/day) creates intestinal discomfort, linked mainly to gut fermentation, in healthy subjects and even more in patients having inflammatory bowel syndrome (Major et al., 2017). This “fructose intolerance” has been attributed to the fact that fructose is poorly digested, and can thus be fermented in the colon (Fedewa & Rao, 2014). It is well recognized that gut microbiota is considered as an environmental factor involved in the control of metabolic alterations and inflammatory disorders (Delzenne & Cani, 2011; Sonnenburg & Backhed, 2016). This bacterial community plays a pivotal role in human nutrition and health by promoting the supply of nutrients, preventing pathogen colonization and shaping and maintaining normal mucosal immunity (Baumler & Sperandio, 2016). Exciting research is now starting to unravel how the composition of the microbiota can offer either resistance or assistance to invading pathogenic species (Baumler & Sperandio, 2016).

Nutritional strategies are of growing interest as a promising tool to shape gut microbial composition, in order to improve key physiological functions in the gut, and beyond (Carmody et al., 2015; David et al., 2014). Dietary fibres, one of the most important classes of compounds in cereal grains play an important role in the control of several metabolic disturbances clustered in the metabolic syndrome (Delzenne & Cani, 2005; Fardet, 2010; Neyrinck & Delzenne, 2010). Wheat bran is a major source of dietary fibres, such as non-starch polysaccharides and lignin. Arabinoxylan (AX) is the most abundant dietary fibre in wheat bran (Broekaert et al., 2011; Neyrinck & Delzenne, 2010) and accounts for 20–30% of dry wheat bran mass, or 70% of its non-starch polysaccharides (Hemdan et al., 2016; Maes & Delcours, 2002). It is a polymer with a D-xylose backbone linked with L-arabinose. Wheat bran contains also several phytochemicals such as ferulic acid, which is the most abundant phenolic compound of the whole-grain (Vitaglione et al., 2015). It is well known that ferulic acid may be involved in anti-inflammatory effects of wheat bran or whole wheat grain demonstrated in humans (Anson et al., 2009; Mateo Anson et al., 2011; Mateo Anson, van den Berg, Havenaar, Bast, & Haenen, 2008; Vitaglione et al., 2015). Moreover, wheat bran fibre can deliver phenolic compounds into the lower gut, and the slow and continuous release of ferulic acid by the action of gut microbiota metabolism may increase circulating ferulic acid and its metabolites, thus providing an amelioration of subclinical inflammation and the long-term benefits associated with whole-grain consumption (Vitaglione et al., 2015). It is thought that processing may release bound phytochemicals from grains but the concentration and extractability of phytochemicals in relation to the exposed surface area is not well documented. However, one study demonstrated that wheat bran particle size influenced on phytochemical extractability and antioxidant properties (Brewer, Kubola, Siriamornpun, Herald, & Shi, 2014). Furthermore, particle size influences the physiological effects of wheat bran such as colonic fermentation in humans (Jenkins et al., 1999). Our previous study has demonstrated that particle size

differently affects adiposity, inflammatory markers and gut microbiota composition in mice (Suriano et al., 2017). Possible beneficial effects of wheat bran fractions on gut microbiota and inflammation have never been investigated in a model of fructose overconsumption. In addition, the contribution of high fructose content to gut microbiota and gut dysfunction remains poorly explored. Therefore, the objective of this study was to evaluate in a model of fructose-induced gut dysfunction, the effects of two wheat bran fractions characterized by different particle sizes on specific gut bacteria known to be involved in the regulation of the gut barrier function and/or inflammatory processes, and to unravel how these effects may affect inflammation outside the gut.

2. Materials and methods

2.1. Animals and diet intervention

Twenty-four male C57BL6 mice (9 weeks old at the beginning of the experiment, Janvier Laboratories, France) were housed in specific pathogen free (SPF) condition in groups of 3 mice per cage in a controlled environment (12-h daylight cycle) with free access to food and water. After one week of acclimatisation, mice were divided in 4 groups ($n = 6/\text{group}$): a control group (CT) fed a standard diet (AIN93M, Research Diets®, composition presented in [Supplementary Table 1](#)) without supplementary sugar, a group fed a standard diet with 30% w/v of fructose (D-fructose high purity grade, VWR) in the drinking water (F group), a group fed a standard diet supplemented with 5% unmodified wheat bran (average particle size of 1690 μm) and with fructose in the drinking water (F + WB group), and a group fed a standard diet supplemented with 5% wheat bran with small particle size (average particle size of 150 μm) and with fructose in the drinking water (F + WBs group). Commercial coarse wheat (*Triticum aestivum* L.) bran was obtained from Dossche Mills (Deinze, Belgium) and it was reduced in particle size with a Cyclotec 1093 Sample mill (FOSS, Höganäs, Sweden) as previously described (Jacobs, Hemdan, Dornez, Delcours, & Courtin, 2015). The composition of the wheat bran materials including soluble fibre and insoluble fibre has been detailed in [Supplementary Table S1](#). The free ferulic acid contents of the standard diet supplemented with WB and WBs were 2.4 mg/kg and 6.8 mg/kg, respectively whereas their Oxygen Radical Antioxidant Capacity (ORAC) were 1.98 and 2.94 mg Trolox equivalents/g, respectively (data obtained from the accredited analytical laboratory Celabor, Belgium). Food intake and water intake were recorded twice a week, and drinking water (with or without fructose) was changed twice a week. The total caloric intake was obtained by multiplying total food and water intake (g) for 3 mice per cage ($n = 2$) by the caloric value of the diet and fructose, i.e. 3.85 kcal/g and 4.00 kcal/g for CT and F groups, respectively, and by assuming that the mice inside the cage ate and drank the same quantities. The caloric value of the standard diet supplemented with cereal fractions was calculated considering that wheat bran fractions were completely processed by the gut microbiota into SCFA available to the host and that the maximal caloric intake through this process would be 2 kcal/g on average. After 8 weeks of dietary treatment and a 6-h period of fasting, mice were anaesthetised with isoflurane (Forene®, Abbott, Queenborough, Kent, England) before exsanguination and tissue sampling. Vena cava blood was collected in EDTA tubes and plasma was immediately collected after centrifugation (13,000g, 3 min) and stored at -80°C . Mice were killed by cervical dislocation. Liver, white adipose tissues (visceral, epididymal and subcutaneous), and gut segments (from ileum, proximal colon and caecum) were carefully dissected, weighed and immersed in liquid nitrogen before storage at -80°C . Animal experiments were approved and performed in accordance with the guidelines of the local ethics committee. The ethical code is 2014/UCL/MD/022. Housing conditions were as specified by the Belgian Law of 29 May 2013, on the protection of laboratory animals (Agreement LA 1230314).

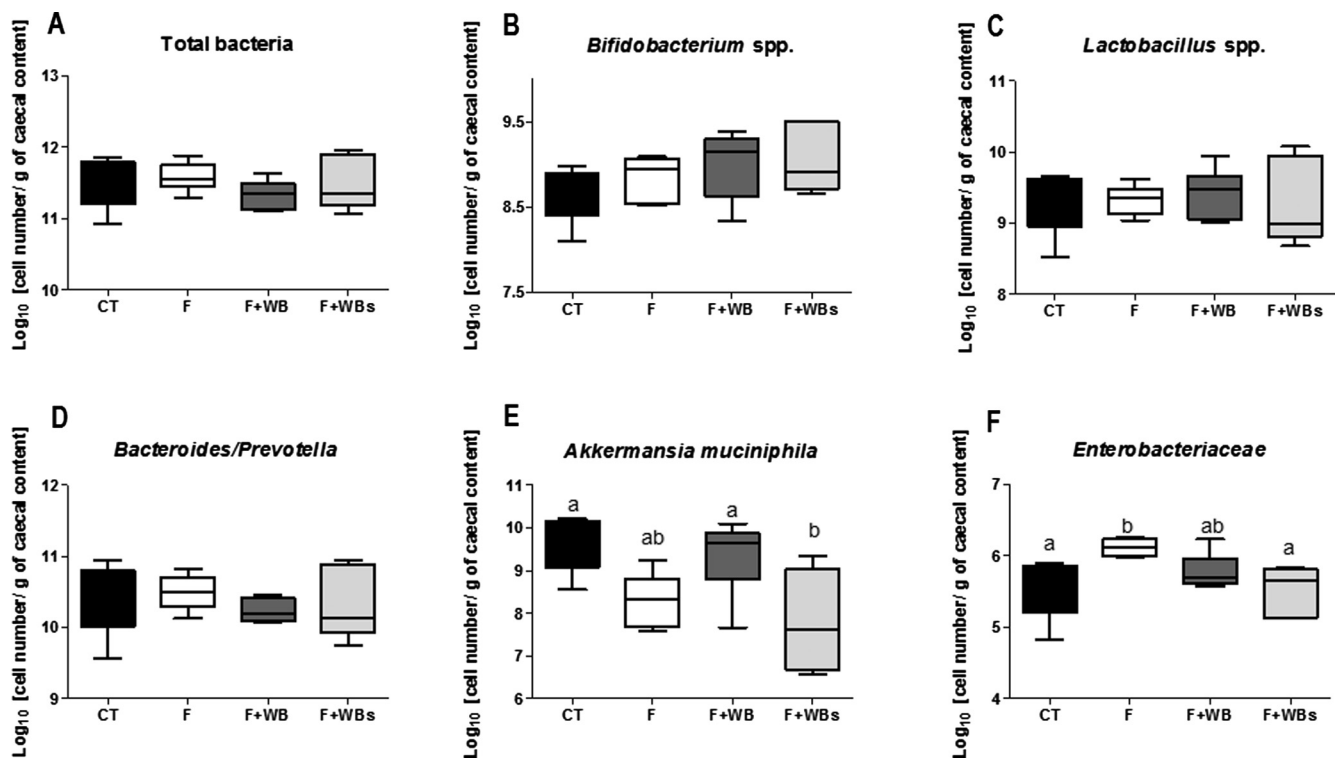


Fig. 1. Impact of the two wheat bran fractions on caecal microbiota assessed by qPCR. Total bacteria (A), *Bifidobacterium* spp. (B), *Lactobacillus* spp. (C), *Bacteroides/Prevotella* spp. (D) *Akkermansia muciniphila* (E) *Enterobacteriaceae* (F) in the caecal content of mice fed a control diet (CT), a control diet with fructose 30% w/v in the drinking water (F), a control diet supplemented with 5% of wheat bran and fructose 30% w/v in the drinking water (F + WB) or a control diet supplemented with 5% of wheat bran with small particle size and fructose 30% w/v in the drinking water (F + WBs) for 8 weeks. Data are Whiskers plots with minimum and maximum. Data with different superscript letters are significantly different at $p < .05$ (ANOVA).

2.2. Analysis of the gut microbiota

Genomic DNA was extracted from the caecal content using a QIAamp DNA Stool Mini Kit (Qiagen, Germany), including a bead-beating step. The composition of the gut microbiota was analysed by quantitative PCR (primers presented in [Supplementary Table S2](#)) as previously described ([Bindels et al., 2016](#)).

2.3. Real-time quantitative PCR

Total RNA was isolated from tissues using the TriPure isolation reagent kit (Roche Diagnostics, Penzberg, Germany). Complementary DNA was prepared by reverse transcription of 1 µg total RNA using the Kit Reverse Transcription System (Promega, Madison, WI, USA). Real-time PCR was performed with a CFX96 Touch Real-Time PCR Detection System and software (Biorad Laboratories Ltd, UK) using SYBR Green (Applied Biosystems, Den Ijssel, The Netherlands and Eurogentec, Seraing, Belgium) for detection. All samples were run in duplicate in a single 96-well reaction plate, and data were analysed according to the $2^{-\Delta\Delta CT}$ method. The purity of the amplified product was verified by analyzing the melting curve performed at the end of amplification. The ribosomal protein L19 (RPL19) gene was chosen as a reference gene. Primer sequences are presented in [Supplementary Table S2](#).

2.4. Biochemical analysis

Concentration of TNFα, interleukin 1β (IL1β), IL6 and monocyte chemotactic protein 1 (MCP1) were determined in 50 µl of plasma using a multiplex immunoassay kit (Bio-Plex Cytokine Assay, Bio-Rad, Nazareth, Belgium) and measured using Luminex technology (BioPlex, Bio-Rad). Total lipid content were measured in the liver tissue after extraction with chloroform-methanol (2:1) according to the Folch method ([Folch, Lees, & Sloane Stanley, 1957](#)), as previously described

([Suriano et al., 2017](#)). Reactive oxygen species (ROS) and lipid peroxidation in the liver tissue (evaluated by measuring thiobarbituric acid-reactive substances (TBARS)) were determined as previously described ([Neyrinck, Etxeberria, et al., 2017](#)). Alanine aminotransferase (ALAT) levels were measured in the serum as markers of liver damage using the ALAT/GPT kit as described by the manufacturer (DiaSys Diagnostic and Systems). All samples were run in duplicate.

2.5. Statistical analysis

Data are presented as mean ± SEM. Statistical significance between groups was assessed using one-way ANOVA. The statistically significant ANOVA tests were followed by post hoc Tukey's multiple comparison tests using GraphPad Prism software (version 5.00, GraphPad Software, San Diego, California, USA). Within-groups variances were compared using a Bartlett's test. If variances were significantly different between groups, values were normalized by Log-transformation before proceeding to the analysis. Differences between groups were assessed using one-way ANOVA, followed by the Tukey post hoc test. Data with different superscript letters were significantly different ($p \leq .05$) according to the post hoc ANOVA statistical analysis.

3. Results

3.1. Both wheat bran fractions induces differential and specific changes in gut bacteria

The fructose intake via the drinking water was not significantly affected by the wheat bran fractions (1.51 ± 0.05 , 1.33 ± 0.06 and 1.28 ± 0.08 g/mouse/day for F, F + WB and F + WBs respectively; $p > .05$, ANOVA) but it is important to note that the total caloric intake of mice in F, F + WB and WBs groups increased after 8 weeks of

treatment, as compared to the CT group. However, the final body weight and fat mass were unaffected by dietary treatments (Supplementary Fig. S1). The weight of the caecal content or caecal tissue was not influenced by the dietary treatments (Supplementary Fig. S1). Some bacteria known to be involved in the regulation of gut barrier function and/or inflammatory processes such as *Bifidobacterium* spp., *Lactobacillus* spp., *Bacteroides/Prevotella* group, *Enterobacteriaceae* and *Akkermansia muciniphila* were analysed in the caecal content by qPCR (Fig. 1). Among those bacteria, the number of *Akkermansia muciniphila* was reduced by about 10-fold after fructose supplementation as compared to the CT group, although this effect did not reach statistical significance (Fig. 1E). Surprisingly, WBs significantly decreased its abundance as compared to the F + WB and the CT groups. Moreover, WBs counteracted the increase in *Enterobacteriaceae* numbers induced by the fructose supplementation whereas WB supplementation had no significant effect (Fig. 1F). In contrast, no significant differences were observed for total bacteria, *Bifidobacterium* spp., *Lactobacillus* spp., *Bacteroides/Prevotella* spp. numbers whatever the dietary treatments (Fig. 1A–D).

3.2. Wheat bran fractions do not affect markers involved in gut barrier function

We measured the expression of secreted antimicrobial peptides (AMP) produced by Paneth cells and/or enterocytes in the ileum and in the colon: C-type lectin, primarily the regenerating islet-derived 3-gamma (RegIIIγ), phospholipase A2g2 (Pla2g2), and lysozyme C (Lys). However, AMP expression was not modified by fructose or wheat bran supplementation (Table 1, Supplementary Table S3). In addition, other markers involved in gut barrier function such as tight junction proteins (claudin 3, occludin, ZO1) and mucin 2 (MUC2) were not modified in the ileum and colon whatever the dietary treatment (Table 1, Supplementary Table S3). In accordance with these results, the occludin protein level in the ileum measured by western blotting analysis was not different between the groups (Supplementary Fig. S2). Altogether, these data suggest that, in our experiment, fructose alone or in combination or not with wheat bran fractions did not alter gut permeability in the ileum or the colon.

3.3. Wheat bran fraction with small particle size improves inflammatory state induced by fructose

Because fructose has been reported to induce intestinal inflammation, we analysed the expression of several macrophage markers (CD11c, CD11b), lymphocyte markers (CD3γ, Foxp3), pattern recognition receptors (TLR2, TLR4) and inflammatory cytokines/chemokines (IL6, IL1β, MCP1, TNFα, IFNγ, IL17A, IL22) in the ileum and/

or the colon (Fig. 2A and Supplementary Table S3). None of those markers were significantly changed whatever the dietary treatments except for IL17A whose expression was significantly decreased in the ileum upon supplementation with both wheat bran fractions. The lower expression of this cytokine was accompanied by a downregulation of IL22 due to bran fractions (9.08 ± 4.82 , 0.65 ± 0.42 , 0.82 ± 0.28 , for F, F + WB and F + WBs, respectively; $p > .05$ ANOVA) suggesting a downregulation of T helper 17 (Th17) cell lineage inside the ileal tissue. In contrast to what happened in the gut, fructose supplementation significantly increased the expression of key inflammatory genes in the liver (CD11c, MCP1, TNFα) as compared to the CT group (Fig. 2B). Importantly, WBs was the sole wheat bran fraction able to significantly reduce the expression of several key inflammatory markers.

Then, we analysed the systemic inflammatory status through measurement of IL1β, IL6, MCP1 and TNFα in the plasma. Again, WBs was the sole dietary treatment able to reduce significantly the circulating level of these cytokines/chemokines upon high fructose intake (Fig. 3).

3.4. Wheat bran fraction with small particle size slightly decreases oxidative stress in the liver without affecting fat accumulation in the liver

We evaluated some markers of hepatic oxidative stress such as TBARS, ROS and the expression of NADPH oxidase 1 (*Nox1*) in the liver tissue (Table 2). It is worth noting that the WBs group exhibited the lowest values for all of these markers (significantly versus the CT group for the ROS content). Moreover, WBs mice exhibited lower level of ALAT in the plasma as compared to the fructose supplemented-mice, suggesting a lower liver injury but these changes were not significant. Fructose over-consumption slightly induced fat infiltration in the liver tissue (Supplementary Fig. S3). Both wheat bran fractions did not significantly affect total lipid content in the liver. This observation was confirmed by histological analysis after oil red O staining (Supplementary Fig. S3).

3.5. Changes in the specific gut bacteria induced by wheat bran fractions correlated with a key hepatic inflammatory parameter

Spearman's correlation tests were performed to evaluate the potential links between caecal bacteria and host parameters among the four experimental groups. The most relevant correlations are shown in Fig. 4. We found that MCP1 mRNA in the liver – which is the marker whose expression is the most significantly affected by the dietary treatments (fructose and WBs) – was negatively correlated with *Akkermansia muciniphila* and positively associated with *Enterobacteriaceae*.

4. Discussion

In this study, we evaluated the potential health benefits of two well-characterized wheat bran fractions, in relationship with their impact on the gut microbiota, in a mouse model of liver inflammation induced by high fructose intake.

It has been previously described that adding sugars to the diet changes the gut microbiota composition (Di Luccia et al., 2015; Ferrere et al., 2016; Magnusson et al., 2015). Those studies showed that sugar consumption significantly elevated gram-negative Proteobacteria, and more specifically within this phylum, microbes from *Enterobacteriaceae* family were increased by added-sugar consumption (Ferrere et al., 2016; Noble et al., 2017). The increase in Proteobacteria is generally associated with gut dysbiosis and altered composition of the gut microbiota but also with an increased intestinal permeability and metabolic endotoxemia (Cani et al., 2007, 2008, 2009; Shin, Whon, & Bae, 2015). In our study, high-fructose intake increased the abundance of *Enterobacteriaceae*, confirming previous results obtained by Ferrere et al. (2016). Interestingly, only WBs blunted the increase of this bacterial family whereas *Akkermansia muciniphila* was increased only upon WB supplementation. Such difference between the two fractions was

Table 1
Gene expression in the ileum.

	F	F + WB	F + WBs
<i>Antimicrobial peptides</i>			
Reg3γ	0.65 ± 0.14	0.77 ± 0.26	0.46 ± 0.14
Pla2g2	1.24 ± 0.27	1.02 ± 0.16	1.12 ± 0.18
Lyz	0.84 ± 0.09	0.90 ± 0.04	1.11 ± 0.21
<i>Gut permeability markers</i>			
Claudin3	1.05 ± 0.27	1.10 ± 0.17	0.91 ± 0.06
Occludin	0.90 ± 0.20	1.23 ± 0.19	1.08 ± 0.10
Zo1	1.08 ± 0.19	1.23 ± 0.17	1.16 ± 0.08
Muc2	1.54 ± 0.66	1.48 ± 0.45	1.02 ± 0.13

Mice were fed a control diet (CT), a control diet with fructose 30% w/v in the drinking water (F), a control diet supplemented with 5% of wheat bran and fructose 30% w/v in the drinking water (F + WB) or a control diet supplemented with 5% of wheat bran with small particle size and fructose 30% w/v in the drinking water (F + WBs) for 8 weeks. Results are expressed as mean ± SEM. Values are expressed as relative units with the mean of CT mice values set at 1; $p > .05$ (ANOVA).

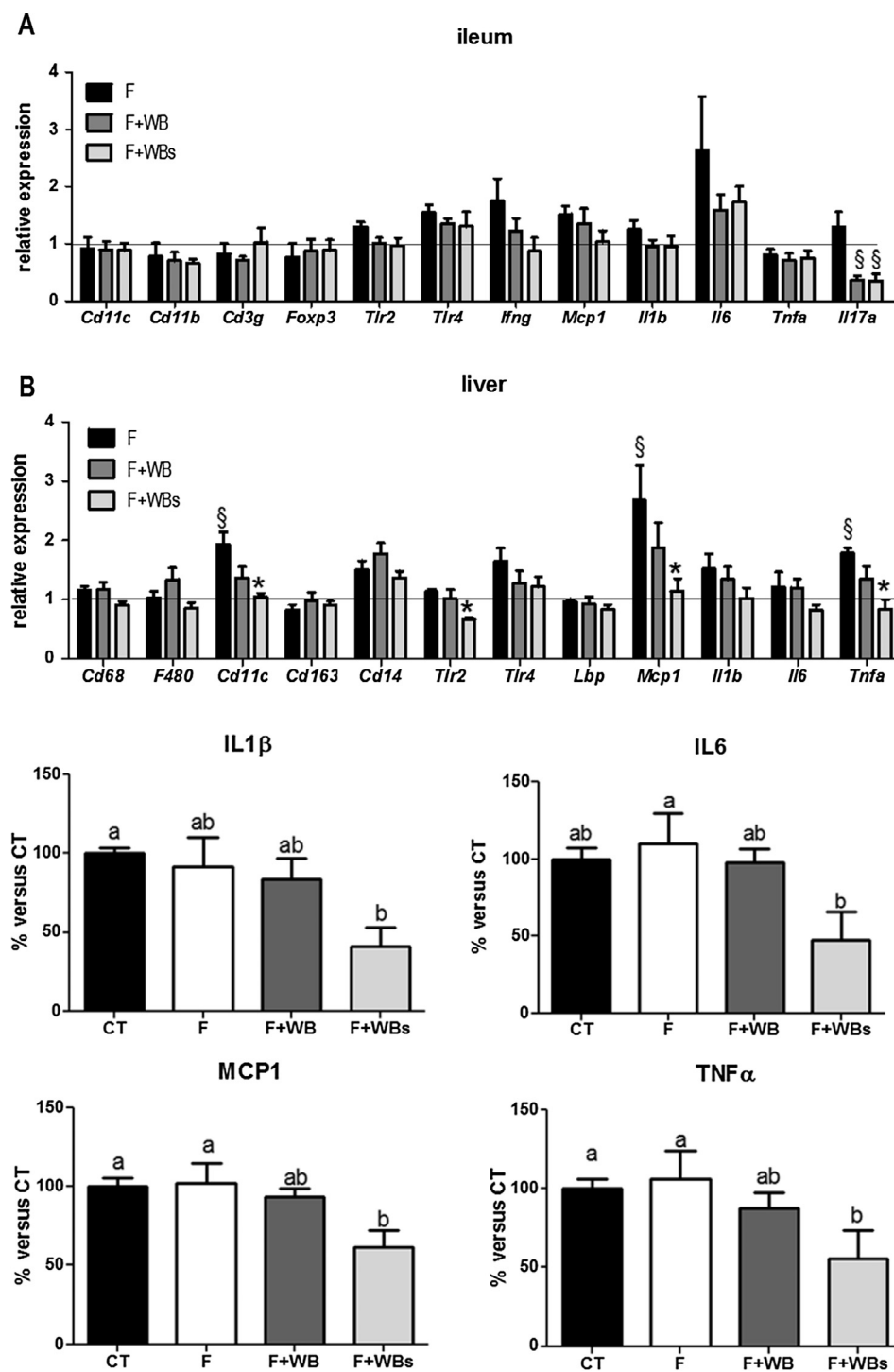


Fig. 2. Impact of the two wheat bran fractions on expression of inflammatory genes in the ileum (A) and the liver (B). Mice were fed a control diet (CT), a control diet with fructose 30% w/v in the drinking water (F), a control diet supplemented with 5% of wheat bran and fructose 30% w/v in the drinking water (F + WB) or a control diet supplemented with 5% of wheat bran with small particle size and fructose 30% w/v in the drinking water (F + WBS) for 8 weeks. Results are expressed as mean \pm SEM. Values are expressed as relative units with the mean of CT mice values set at 1. § p < .05 versus CT; * p < .05 versus F (ANOVA).

Fig. 3. Impact of the two wheat bran fractions on systemic inflammatory parameters. Mice were fed a control diet (CT), a control diet with fructose 30% w/v in the drinking water (F), a control diet supplemented with 5% of wheat bran and fructose 30% w/v in the drinking water (F + WB) or a control diet supplemented with 5% of wheat bran with small particle size and fructose 30% w/v in the drinking water (F + WBS) for 8 weeks. Results are expressed as percentage of CT mice (mean \pm SEM). Data with different superscript letters are significantly different at p < .05 (ANOVA).

already observed in a model of high fat-induced obesity: only the crude fraction of wheat bran (WB) distinguished from fraction with small particle size (WBS) by the capacity to increase the *Akkermansia* genus (Suriano et al., 2017). Interesting papers show, *in vitro* on Caco-2 cells, that specific bacteria belonging to *Bifidobacterium* and *Lactobacillus* and used as probiotics are able to compete with *Enterobacteriaceae* for enterocyte adhesion (Candela et al., 2008; Lee et al., 2000). Even if we do not have any changes in lactobacilli and bifidobacteria in wheat bran-treated mice, we cannot exclude that changes in bacterial environment could also contribute to the improvement of intestinal functions by wheat bran fractions. Previous studies in mice showed that changes in the gut microbiota in favor of *Akkermansia muciniphila* or oral

administration of this bacterium improved gut barrier, metabolic parameters and inflammatory disorders (Everard et al., 2013, 2011, 2014; Plovier et al., 2017). In our study, WB did not exert hepatic anti-inflammatory effects or changes of several markers in the ileum or the colon regarding the gut barrier function but we observed a lower expression of IL17A in the ileum (and IL22 to a lower extent). Those cytokines were also downregulated by the WBS fraction and may potentially reflect Th17 cell inhibition (Sabat, Ouyang, & Wolk, 2014). Indeed, Th17 cells belong to a recently identified T helper subset, in addition to the traditional Th1 and Th2 subsets, and are characterized as preferential producers of IL17A and IL22 (Ouyang, Kolls, & Zheng, 2008). It is worth mentioning that the effector cytokines of Th17 cells

Table 2
Parameters related to oxidative stress and liver injury.

	CT	F	F + WB	F + WBs
<i>Plasma</i>				
ALAT (U/L)	7.34 ± 1.43	11.13 ± 0.46	11.10 ± 2.93	7.99 ± 0.82
<i>Liver</i>				
ROS content (%)	100 ± 4 ^a	87 ± 8 ^{ab}	79 ± 5 ^{ab}	68 ± 7 ^b
RFU/μg protein)				
TBARS content (nmol/μg protein)	132 ± 20	108 ± 6	100 ± 9	102 ± 15
<i>Nox1</i> mRNA (relative expression)	1.00 ± 0.08	1.11 ± 0.16	1.11 ± 0.15	0.84 ± 0.06

Mice were fed a control diet (CT), a control diet with fructose 30% w/v in the drinking water (F), a control diet supplemented with 5% of wheat bran and fructose 30% w/v in the drinking water (F + WB) or a control diet supplemented with 5% of wheat bran with small particle size and fructose 30% w/v in the drinking water (F + WBs) for 8 weeks. Results are expressed as mean ± SEM. Data with different superscript letters are significantly different at $p < .05$ (ANOVA). ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; ALAT, alanine aminotransferase.

play indispensable roles in tissue immunity mediating host defense mechanisms to various infections, especially extracellular bacterial infections (Ouyang et al., 2008). However, the role of IL17A in gastrointestinal inflammation remains controversial (Yamada et al., 2011) and further studies are needed to better understand the Th17 cell response to wheat bran supplementation in the mouse model of high-fructose intake inducing liver inflammation.

In our setting, we were unable to highlight a loss of the tight junction proteins in any part of the intestine. In addition to this lack of inflammatory events in the ileum or the colon, fructose consumption did not change body weight and the amount of the fat mass. The same observations have been previously described in mice with free access to solutions containing either 30% or 15% of fructose in the drinking water for 8 or 12 weeks of treatment respectively (Baena et al., 2017; Bergheim et al., 2008). It is well known that fructose consumption increases oxidative stress (Lim et al., 2010; Zhang et al., 2017). ROS and TBARS overproduction, a common metric of oxidative stress and lipid peroxidation, are considered as important mediators in the development of liver damage (Dryden, Deaciuc, Arteel, & McClain, 2005). ROS can activate several intracellular signalling pathways that can lead to hepatocyte apoptosis (Gambino, Musso, & Cassader, 2011). In our study, neither ROS nor TBARS contents were increased in the liver of fructose-fed mice but the lowest level of ROS was observed upon WBs consumption together with a decrease of ALAT activity in the serum of F + WBs (versus the F group), suggesting a reduction in hepatic

oxidative stress and injury due to WBs supplementation.

Gut microbes dialogue with the host via specific cell membranes or related molecules that may activate pattern recognition receptors such as TLRs (Beutler, 2004). The interaction between microbial components and TLRs, which are innate immune sensors present on certain immune cells within the gut, contributes to maintenance of the mucosal and systemic immune status (Thaiss, Zmora, Levy, & Elinav, 2016). As shown by other authors, chronic fructose exposure induces translocation of bacterial components in the liver, and primes hepatic Kupffer cells, thereby leading to inflammatory response, cytokine production and chemokine-mediated recruitment of acute inflammatory cells (Beutler, 2004; Spruss et al., 2009). The activation of the resident macrophages in the liver is a key event contributing to NAFLD (Lanthier et al., 2010; Neyrinck et al., 2009).

In the present study, and in contrast to what happened in the distal part of the gut, several inflammatory markers were induced by high-fructose intake in the liver tissue. Interestingly, WBs was the sole fraction that blunted hepatic inflammation by normalizing the macrophage infiltration in this tissue through the significant downregulation of CD11c, MCP1 and TNF α reflecting the M1 macrophage phenotype. Moreover, supplementation with WBs inhibited also the expression of TLR2 which confirms a modulation of the hepatic immune response. The concentrations of soluble fibre calculated in the diets supplemented with WB and WBs were 2.35% and 2.34%, respectively, whereas the concentrations of insoluble fibre were 0.32% and 0.38% suggesting that the anti-inflammatory effects observed with WBs might not be attributed to the type of fibre in the fraction. It is worth to note that both the free ferulic acid content of the diet supplemented with WBs and its antioxidant capacity (ORAC) were higher than their levels in the diet supplemented with WB. It is well known that ferulic acid from aleurone determined the antioxidant potency of wheat grain and may be involved in anti-inflammatory effects of wheat bran or whole wheat grain demonstrated in humans (Mateo Anson et al., 2008, 2011; Vitaglione et al., 2015). This leads us to postulate that the higher free ferulic acid content and the higher antioxidant capacity obtained in the diet containing the WBs fraction could participate in the anti-inflammatory effects observed in the liver of WBs-treated mice. Interestingly, *Enterobacteriaceae* and *Akkermansia muciniphila* were correlated with MCP1, a proinflammatory marker that was highly affected by the supplementation with both fructose and WBs. The fact that WBs, the only bran fraction to significantly affect hepatic and systemic inflammation, decreased the *Enterobacteriaceae* levels without affecting *Akkermansia muciniphila* one, suggest that the decrease in *Enterobacteriaceae* could play a key part in the anti-inflammatory effects of WBs. It is important to note that WBs decreased inflammatory and oxidative stresses in the liver without affecting significantly fat accumulation in this tissue. Indeed, the mice treated with WB or WBs had

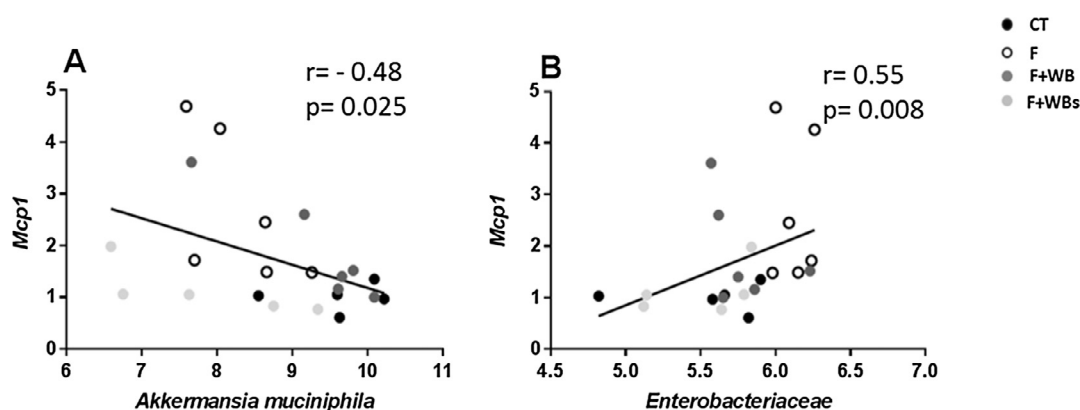


Fig. 4. Spearman correlation analysis between the hepatic expression of MCP1 and two caecal bacteria significantly affected by the dietary treatments (*Akkermansia muciniphila* and *Enterobacteriaceae*). Mice were fed a control diet (CT), a control diet with fructose 30% w/v in the drinking water (F), a control diet supplemented with 5% of wheat bran and fructose 30% w/v in the drinking water (F + WB) or a control diet supplemented with 5% of wheat bran with small particle size and fructose 30% w/v in the drinking water (F + WBs) for 8 weeks.

not significant effect on liver lipid content or on adiposity. Therefore, in this case, we cannot see any effect related to the fat binding capacity of wheat bran that we have previously shown in a model of western diet fed obese mice (Suriano et al., 2017). Interestingly, this study revealed that WBs significantly reduced systemic inflammatory parameters as confirmed by the reduction of four important proinflammatory cytokines/chemokines. Altogether, our data led us to postulate that the management of hepatic and systemic inflammation by WBs occurred independently of any significant changes in lipid accumulation and independently of barrier alterations or immune function in the distal part of the gut where fructose fermentation occurred. In humans, the relationship between small intestinal bacterial overgrowth and liver inflammation linked to non-alcoholic fatty liver disease has been described, and nutritional tools like wheat bran extract could be interesting in this particular context also (Kapil et al., 2016). Particle size is another factor to be considered while considering functionality of wheat bran in cereal products. Indeed, it influences the physico-chemical properties and the physiological effects of wheat bran such as colonic fermentation in humans and affects adiposity, inflammatory markers and gut microbiota composition in mice (Jenkins et al., 1999; Suriano et al., 2017). In this respect, the size of particles may be fundamental because it may influence the fermentability of dietary fibre as well as the availability of bound polyphenols (ferulic acid) to act as antioxidants along the gastro-intestinal tract and to be released by the intestinal microbial communities (Brewer et al., 2014; Onipe, Jideani, & Beswa, 2015; Vitaglione, Napolitano, & Fogliano, 2008). These factors might have relevant implications in the systemic and hepatic anti-inflammatory effects observed only with WBs.

In conclusion, our findings support the interest of the ingestion of some wheat bran fraction - characterized by a small particle size- in the management of inflammatory disorders outside the gut, namely in the liver. We propose that the physicochemical characteristics of the bran fractions may contribute to selective changes in the gut microbiota, allowing to counteract the bloom in *Enterobacteriaceae*, associated with liver inflammation.

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Conflict of interest

None.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jff.2017.12.035>.

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